STUDIA FORESTALIA SUECICA

Nr 68 1969

Growth of *Fomes annosus* in the precence of host material from Norway spruce and silver fir

Tillväxten hos Fomes annosus odlad på värdväxtmaterial av gran och silvergran

by

ARNE HYPPEL

Department of Forest Botany

SKOGSHÖGSKOLAN
ROYAL COLLEGE OF FORESTRY
STOCKHOLM

Received for publication:
Febr. 5, 1969

ESSELTE AB, STHLM 69

ABSTRACT

Root and stem material of Norway spruce *Picea abies* KARST. and silver fir *Abies pectinata* D.C. were used as substrate for the growth of *Fomes annosus* (FR.) CKE. On material from Norway spruce the root fungus developed without any inhibitions. The silver fir material exerted strong inhibitory effects on the fungus with all treatments used.

Introduction

The root and stem disease on conifers caused by *Fomes annosus* (FR.) CKE. is mainly a biological problem, where basic knowledge concerning the dissemination biology of the fungus still is lacking. The presence of the root rot has, however, consequences for the whole field of silviculture in areas with heavy attacks. The choice of tree species after a clear cut is a crucial point with economic profit or loss in the future. One of the recommendations given for forest practise by RENNERFELT (1946) is to use silver fir, *Abies pectinata* D.C., considered as resistant under Swedish conditions. A stand of this species was planted on Omberg at the lake Vättern sixtyfive years ago and adjacent to it a stand of Norway spruce *Picea abies* KARST. A recently made analysis of the root rot frequency (HYPPEL unpublished) of the two stands showed that 42 % of the Norway spruces were attacked by *F. annosus* but none of the silver firs.

There are, however, some reports on attacks of silver fir by *F. annosus* under special conditions. Young plantations of *A. pectinata* on sites with former stands heavily attacked by the root rot fungus were reported damaged (FERDINANDSEN and JØRGENSEN 1938—1939, LØFTING 1959—1960, PEACE 1962). On calcareous soils BIRAGHI (1963) reported attacks on old silver firs by *F. annosus* in the Apennines. These reports, however, seem to be exceptions from the general experience that *A. pectinata* is less susceptible to attacks of *F. annosus* than any other conifer species of practical importance in Europe.

The experiments reported below concern the comparative growth of *F. annosus* on both root material and root exudates of the two species *A. pectinata* and *P. abies*. This latter phenomenon has been investigated in connection with the rhizosphere research concerning the influence of the root

exudates on the microflora of the root surroundings. Literature reviews in this area have been given by STARKEY (1958), BÖRNER (1960), WOODS (1960), SCHROTH and HILDEBRAND (1964) and BAKER (1968).

Material and methods

1. Sawdust

Fresh root sections were excised from two 40-year old silver firs and two Norway spruces about 0.5 m from the stem, then barked and ground. Fresh stems obtained 0.5 m from the ground were prepared in the same fashion. The ground material was dried for 2 hours at 105°C. Twenty-five ml of ground, unpacked material and 20 ml of autoclaved 1.5 % water agar were poured into Petri dishes. Once solified the dishes were inoculated in the centre with slant culture pieces of *F. annosus*, No. Sä 1. Radial growth of the fungal colony was measured daily in mm, the average from four dishes was calculated and recorded. The incubation was performed at 15° and 25°C.

2. Root extracts

Roots were cut from potted 4-year old Norway spruce and silver fir grown under greenhouse conditions (HYPPEL 1968/66). The roots were then carefully washed and ground in a rapid operation at low temperature. To each 200 g of root material, 200 ml sterile distilled water was added and left for extraction at +4°C during 24 hours. The extracts were sterilized by filtration and used as culture media in 3 concentrations: undiluted, diluted 1 : 5 and 1 : 20 respectively. Controls were supplied with SHIVE's nutrient solution including 1 % glucose, 20 γ aneurine and 10 γ biotine. At least 4 replicate flasks containing 20 ml each were used. Dry mycelial weights were calculated after 30 days culture at 22°C.

3. Root exudates

4-year old plants of *P. abies* and *A. pectinata* of commercial origin were grown in a greenhouse for 3 months. Once a week SHIVE's nutrient solution (PERSSON—HYPPEL 1963) was added beside the common tap water irrigation. Three plants were then removed from the pots after 3, 6, 9 and 12 weeks together with the terralite-substratum and put in a beaker with 500 ml distilled water. After about two hours at +4°C in the dark the solutions were sterilized by filtration and used with the germination test for *F. annosus*

conidia in moist chambers. About 100 000 conidia per ml were prepared by pouring 10 ml of the exudate on a Petri dish containing a 10 day-old culture of *F. annosus* and rubbing the conidiophores with a glass rod. Controls were run in a 0.5 % water solution of malt extract (RENNERFELT 1949).

A modification of the cellophane test for study of the antagonistic effect of soil microorganisms in order to avoid contamination (WASTIE 1961) was used to evaluate for the effect of the exudate on mycelia of *F. annosus*. Plants of Norway spruce and silver fir were removed from the pots and the roots put between moist cellulose screens ("Wettex"). The plants were left 4 days under light and sufficient water supply at 20°C. For harvesting, the used cellulose screens were cut in circular pieces of 90 mm in diameter and placed at the bottom of Petri dishes. Circular cellophane discs, sterilized in 70 % ethyl alcohol and dried were put on the cellulose discs and gently pressed to insure contact. Finally a 2—3 mm thick disc of malt agar 30 mm in diameter, was placed on the cellophane membrane and inoculated at the centre with a small piece of *F. annosus*.

Separation and isolation of the exudates were performed by paper chromatography in cellosolve-pyridine-acetic acid-water (80-40-10-10) ascending at room temperature. Bioassays were performed with conidia of *F. annosus* germinating in the moist chambers in eluates from the paper strips. Controls were prepared as described above.

Results

1. Sawdust experiment

The growth response of *F. annosus* on sawdust from *A. pectinata* and *P. abies* respectively was principally different. On sawdust from spruce the root rot fungus developed normally. On the other hand, when cultured on sawdust from silver fir no growth of *F. annosus* was recorded as can be seen from figure 1.

This difference between the two host species can be interpreted either as a lack of growth promoting substances or presence of inhibitors in the silver fir.

There are some other differences noticeable in figure 1. The growth rate of the root rot fungus was greater on root material of Norway spruce than on material from the stem, which is seldom the case under natural conditions (see discussion). The very strong influence of temperature on the growth rate is remarkable. With a 10°C increase the radius increases 3.5 times during comparable days.

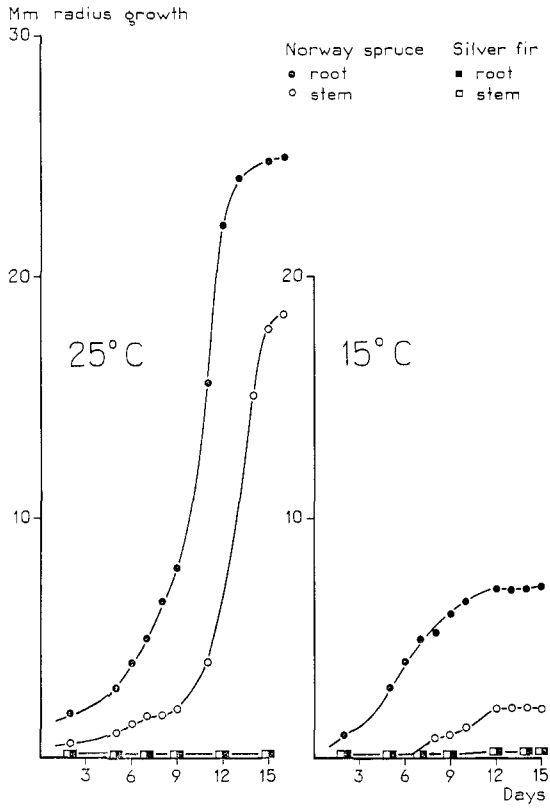

Fig. 1. The radial growth of *Fomes annosus* on sawdust substrate of Norway spruce and silver fir at two temperatures.

2. Root extract experiment

Mycelial growth of *F. annosus* on the wood of silver fir and Norway spruce was similar on the whole to that obtained in sterile water extracts (figure 2).

The different host effects were most obvious at the highest concentration of spruce — fir extracts. No mycelium developed outside the inoculum piece of *F. annosus* in the silver fir extract, while in the spruce extract a measurable mycelial mat was growing. At lower concentrations of spruce extract less mycelium was harvested, probably because of a decreasing supply of necessary nutrients. In silver fir extracts the trend was opposite and small amounts of mycelium developed in the diluted extracts. These results support the assumption, mentioned earlier, that some inhibiting substances influence the growth of *F. annosus* in *A. pectinata*.

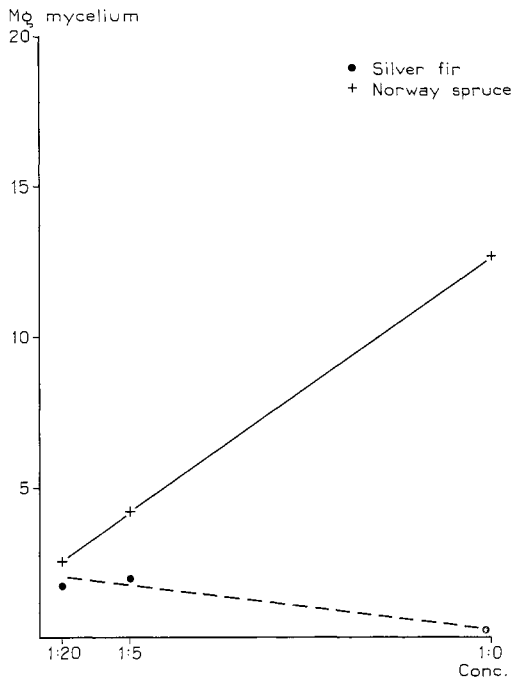

Fig. 2. Mycelial dry weights of *Fomes annosus* in root extracts of Norway spruce and silver fir at 3 concentrations.

In *P. abies* there seemed to be a lack of inhibitors but instead a supply of growth promoting substances. However in this experiment the water extracts did not yield as much mycelia as did the controls with nutrient solution; 12.7 mg in the most concentrated extract and 17.2 mg in SHIVE's solution.

3. Root exudate experiment

The exudates from intact roots were tested with conidia of *F. annosus* in moist chambers and with mycelia in a modified cellophane experiment.

In the table below the germination of conidia is calculated after 24 hours at 20 °C from ten countings in the microscope (200 x). Germination was established when the length of the germtube exceeded the diameter of the conidia.

% germinating conidia			
Silver fir exudate	Norway spruce exudate	0.5 % malt extract	Distilled water
0	58.7	50.5	8.9

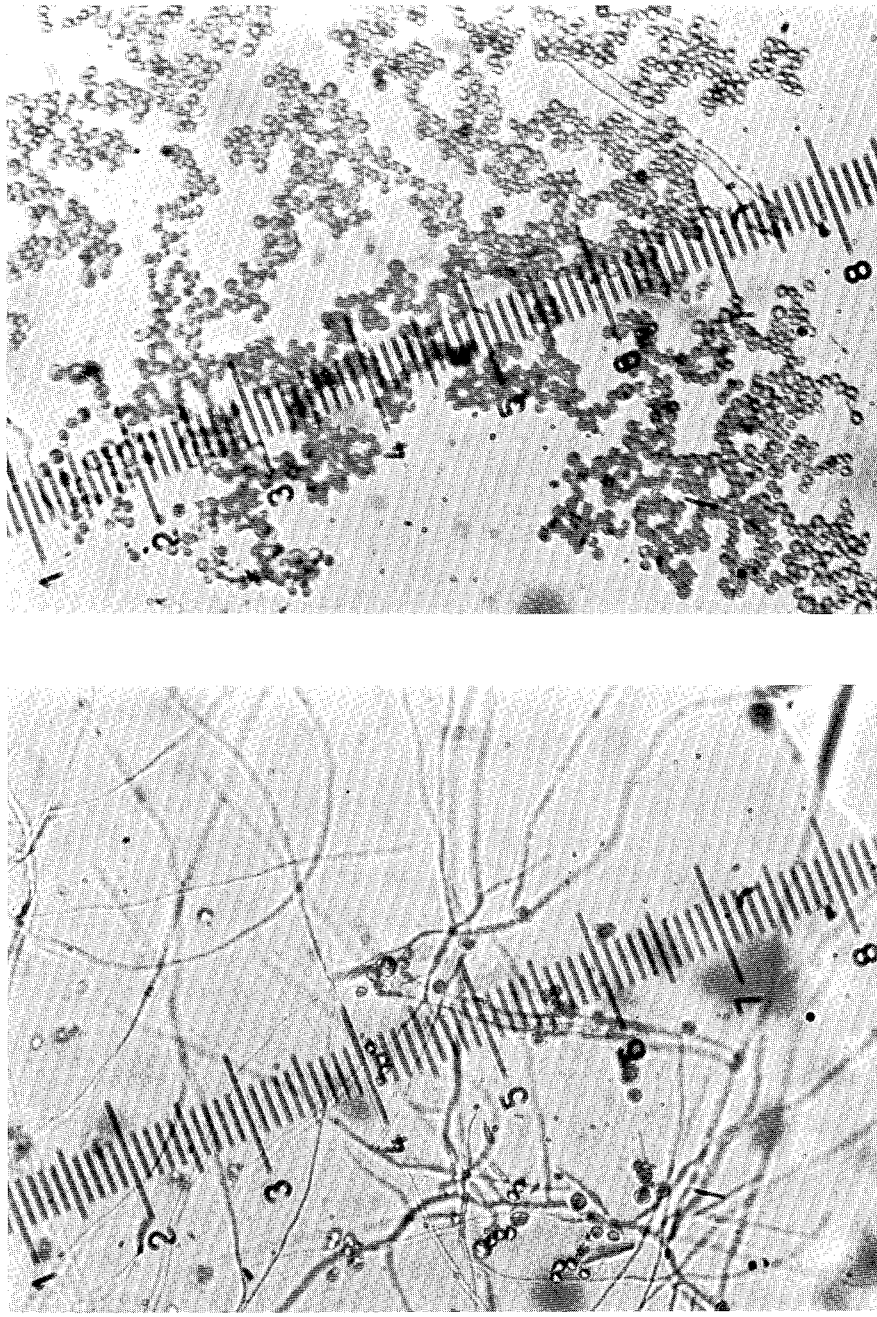

Fig. 3. Conidia of *Fomes annosus* treated with root exudates of silver fir (above, no germination) and Norway spruce (below, abundant germination). 300 x.

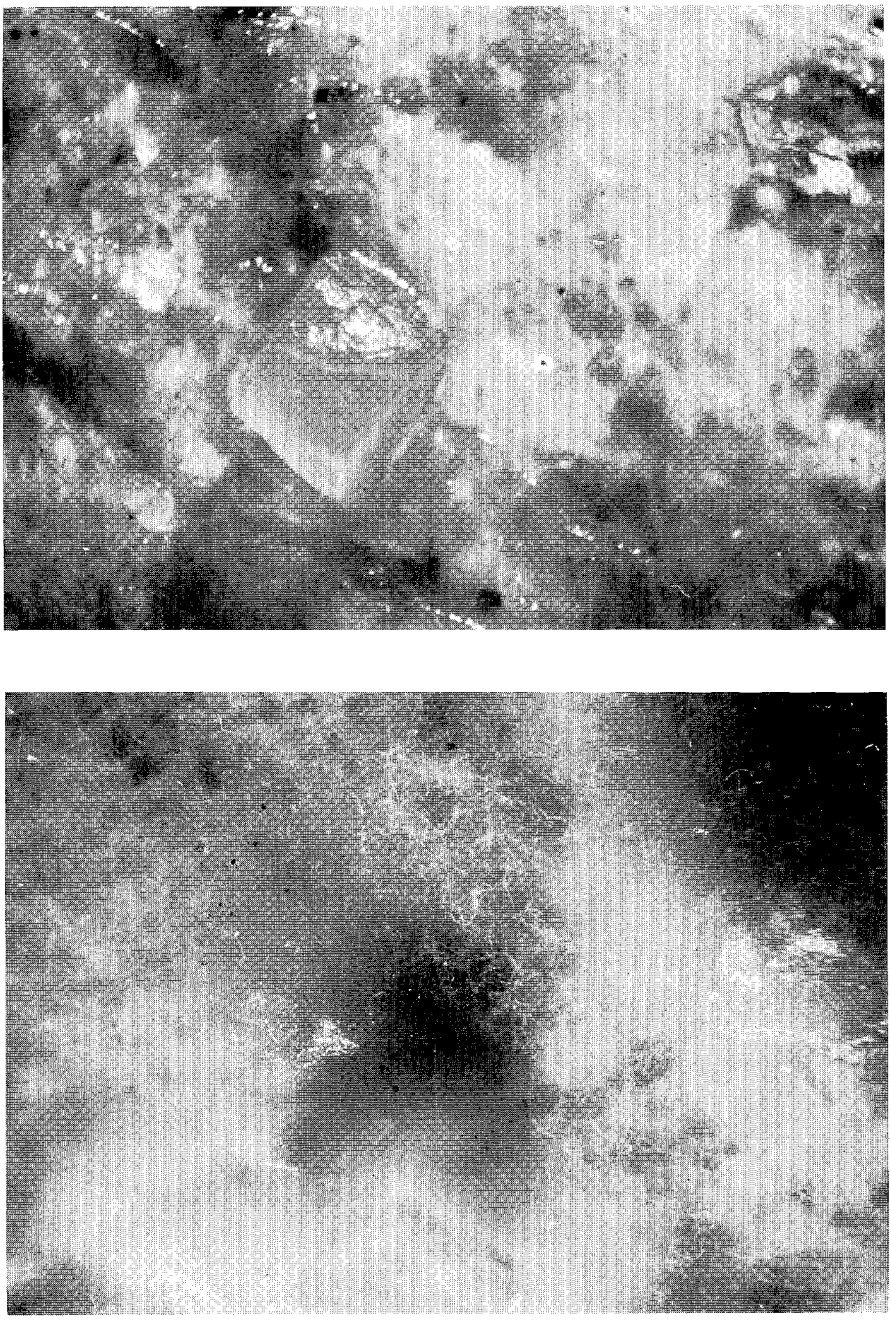

Fig. 4. Mycelial development of *Fomes annosus* in cellophane diffusion test with exudates of silver fir (above, no growth) and Norway spruce (below, superficial hyphae).

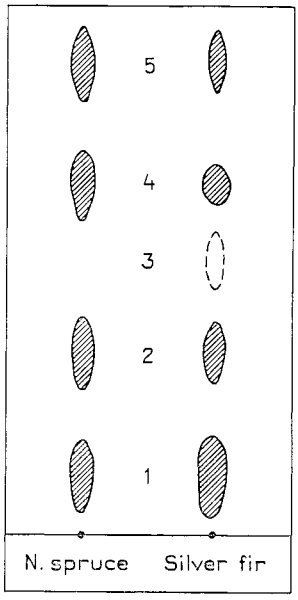

Fig. 5. Spot location of exudates from Norway spruce and silver fir in a paper chromatographical analysis of extracts from silver fir and Norway spruce.

Again the differentiated effect on *F. annosus* of the two conifer species appeared, this time on reproductive organs. With exudates from silver fir no germination at all took place; with exudates from Norway spruce a slight stimulation could be observed compared with controls in malt extract. In distilled water a sparse germination occured (*cf.* RENNERFELT 1949).

The cellophane test of the mycelial growth of *F. annosus* was principally in agreement with that of the conidial germination. The thin mycelial mats developed on the malt agar discs could only be evaluated qualitatively.

In figure 4 the inoculum pieces of *F. annosus* and their close vicinity are photographed. It cannot be excluded that some biologically active substance was exuded from the roots and absorbed by the cellulose discs, diffused through the cellophane film and finally spread into the malt agar disc with effect on the root rot fungus inoculated on the top of it. The root exudate from silver fir exerted a total inhibition of *F. annosus* in contrast to the very slight influence from exudates of Norway spruce. This was repeated at all the different samplings.

4. Paper chromatography experiments

With the hypothesis in mind that some substance inhibitory to *F. annosus* is likely to be present in the silver fir, a rough bioassay with conidia was

performed using paper chromatography separation methods. It was possible to isolate at least five spots on the paper. These were detectable by spraying with a 0.04 % bromcresolgreen solution ranging the pH 3.8—5.4 and indicating an acidic nature of the spot substances. The chromatographed exudates of silver fir and spruce have 4 spots in common, *i.e.* with the same R_f-value and colour. Silver fir has a colour-weak spot (No. 3) which Norway spruce is lacking.

The biological activity of the spots differed. Conidia of *F. annosus* did not germinate when eluates from spot Nos. 2,4 and 5 of silver fir were used as germination medium. A weak inhibition was also recorded from spot No. 1. In Norway spruce, on the other hand only eluate from spot No. 1 had an inhibitory effect on the conidial germination.

Spot No.	% germination	
	Silver fir	Norway spruce
1	10.2	0
2	0	51.0
3	42.5	43.4
4	0	46,7
5	15.5	52.1
Controls	50.5	

Discussion

A difference linked to the host species was shown uniformly in the experiments performed and supported by field observations in Europe regarding the attacks of *F. annosus*. The extensive literature on wood components does not provide a comprehensive comparative study between silver fir and Norway spruce. In a table of wood ash TRENDELENBURG/MAYER-WEGELIN (1955) reported great differences between *P. abies* and *A. pectinata* in the content of CaO (33.6 *versus* 10.0 dry weight percentage), K_2O 3.6 *v.* 40.6 and Na_2O 18.7 *v.* 0,7 % respectively. Corresponding calculations on fats, oils, terpenes, lignin, cellulose and holocellulose were not made. RENNERFELT (1949) reported that such heartwood extracts of spruce as conidendrin and pinoresinol had no significant inhibitory effect on the germination of conidia of *F. annosus*. Recent papers on the host resistance against *F. annosus*, however, mainly concern species of pine. JØRGENSEN (1961) reported formation of pinosylvin, toxic to the root rot fungus, in an injuried sapwood of red pine. GIBBS (1967) found a correlation between resin flow and resistance against *F. annosus* in Scots pine. SHAIN (1967) considered that not only pinosylvins but also phytoalexins acted inhibitory to *F. annosus* in the reaction zone of loblolly pine. COBB *et* al. (1968) extracted some oleoresins from ponderosa pine which inhibited *F. annosus* in bioassay tests.

The superior growth rate of the fungus in sawdust root material of spruce compared with that in stem material was reported by PLATT *et* al. (1965) and discussed on a C : N basis, but not supported by the experiences in the field. JØRGENSEN, LUND and TRESCHOW (1939) and NILSSON and HYPPEL (1968) reported the opposite development with inoculation and by artificial scars in spruce. In the last paper the moisture conditions of root and stem were measured and discussed in relation to the growth of *F. annosus*.

Also in the experiment with exudate a marked inhibition of both conidia and mycelium occurred at all but weak concentrations of inhibitory substances. Some of the extensive literature on plant exudates and rhizosphere phenomena, reviewed by SCHROTH and HILDEBRAND (1964) discusses direct inhibitory effect on root pathogens *in vitro*. REYNOLDS (1964) and TIMONIN (1941) reported that exudates from resistant flax inhibited growth of wilt-causing *Fusarium* spp. BUXTON (1957) showed that exudates from resistant peas depressed conidial germination and mycelial growth of *Fusarium oxysporum* FR. *f. pisi.* (LINF.) SNYDER & HANSEN. On the other hand exudates from susceptible strains stimulated the pathogenic fungus.

The ecological effect of root exudates in the rhizosphere is, however, very complex and obscure as is stressed by SCHROTH and HILDEBRAND (1964). For *F. annosus* it is likely to assume that, beside the stump infection by spores, a direct attack may hit the roots. As MOLIN (1957) showed, spores can penetrate soils without loosing viability. At the landing, occasionally, on the root surface, the conditions prevailing at the rhizosphere may determine the fate of that spore and already at this first confrontation between the host and the pathogen decide resistance or susceptibility.

Summary

The influence of bark-free ground root material, sawdust like, from Norway spruce and silver fir on the root rot fungus *Fomes annosus* was tested in three different ways.

1. Sawdust cultures inoculated with the fungus yielded growth on spruce material but complete inhibition on material of silver fir. The growth was measured as mm of radial growth. The growth rate was higher at 25 °C than at 15 °C. Both growth rate and final development was superior in the root material than in material from stems.
2. Water extracts of ground silver fir and Norway spruce wood were inoculated with mycelium of *F. annosus*. With increasing concentration of extracts from spruce the mycelial yield increased. With extracts from fir a slight increase of mycelium was correlated with decreasing concentrations of extracts suggesting presence of an inhibiting factor, absent in spruce extracts.
3. Exudates from roots of living plants of silver fir and Norway spruce influenced both conidia and mycelia of *F. annosus* in a fashion analogous to that observed in the preceding experiments. Total inhibition was exerted by exudates from silver fir. A paper chromatography analysis with conidial bioassay indicated more spots of inhibitory effect from the silver fir than from the extract of Norway spruce. The chemical background of these differences is unknown.

LITTERATURE CITED

BAKER, R., 1968. Mechanisms of biological control of soil borne pathogens. — Ann. Rev. Phytopath. 6, 263—294.

BIRAGHI, A., 1963. Rassegna dei casi fitopatologici forestali osservati dal 1950 al 1962. — Acc. Ital. Sci. For. 33—109.

BUXTON, E. W., 1957. Some effects of pea root exudates on physiological races of *Fusarium oxysporum* FR. *f. pisi* (LINF.) SNYDER & HANSEN. — Trans. Brit. Mycol. Soc. 40, 145—154.

BÖRNER, H., 1961. Experimentelle Untersuchungen über dei Bodenmüdigkeit beim Apfel (*Pirus malus* L.). — Beiträge zur Biol. d. Pflanzen 36, 97—137.

COBB, F. W. Jr, KRSTIC, M., ZAVARIN, E. and BARBER, H. W. Jr, 1968. Inhibitory effects of volatile oleoresin components on Fomes annosus and four Ceratocystis species. — Phytopath. 58. 1327—35.

FERDINANDSEN, C. and JØRGENSEN, C. A., 1938—1939. Skovtræernes Sygdomme. — Köpenhamn.

GIBBS, J. N., 1968. Resin and the resistance of conifers to *Fomes annosus*. — Ann. Bot. 32. 649—65.

HYPPEL, A., 1968. Effect of *Fomes annosus* on seedlings of *Picea abies* in the presence of *Boletus bovinus*. — Stud. For. Suec. 66, 1—13.

LØFTING, E. C. L., 1959—1960. Danmarks ædelgranproblem. — Det Forstl. Forsøgsv. 26, 1—249.

MOLIN, N., 1957. Om *Fomes annosus* spridningsbiologi. — Medd. Stat. Skogsf.-inst. 47, 1—36.

NILSSON, P. O. och HYPPEL, A., 1968. Studier över rötangrepp i sårskador hos gran. — Sv. Skogsv. Förb. Tidskr. 8, 675—713.

PEACE, T.R., 1962. Pathology of trees and shrubs with special reference to Britain. — Oxford.

PERSSON-HYPPEL, A., 1963. The influence of temperature on the antagonistic effect of *Trichoderma viride* FR. on *Fomes annosus* (FR.) CKE. — Stud. For. Suec. 4, 1—11.

PLATT, W. D., COWLING, E. B. and HODGES, C. S., 1965. Comparative resistance of coniferous root wood and stem wood to decay by isolates of Fomes annosus. — Phytopath. 55. 1347—53.

RENNERFELT, E., 1946. Om rotrötan (*Polyporus annosus* FR.) i Sverige, dess utbredning och sätt att uppträda. — Stat. Skogsf.-inst. 35, 1—88.

— 1949. The effect of soil organisms on the development of *Polyporus annosus* FR., the root rot fungus. — Oikos 1, 65—78.

REYNOLDS, E. S., 1931. Studies on the physiology of plant disease. — Ann. Missouri Bot. Gard. 18, 57—95.

SCHROTH, M. N. and HILDEBRAND, D. C., 1964. Influence of plant exudates on root-infecting fungi. — Ann. Rev. Phytopath. 2, 101—133.

SHAIN, L., 1967. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. — Phytopath. 57. 1034—45.

STARKEY, R. L., 1958. Interrelations between microorganisms and plant roots in the rhizosphere. — Bact. Rev. 22, 154—172.

TIMONIN, M. I., 1941. The interaction of higher plants and soil microorganisms. III. Effect of by-products of plant growth on activity of fungi and actinomycetes. — Soil Sci. 52, 395—417.

TRENDELENBURG, R./MAYER-WEGELIN, H., 1955. Das Holz als Rohstoff — München.

WASTIE, R. L., 1961. Factors affecting competitive saprophytic colonization of the agar plate by various root infecting fungi. — Trans. Brit. Mycol. Soc. 44, 149—159.

WOODS, F. W., 1960. Biological antagonisms due to phytotoxic root exudates. — The Bot. Rev. 26, 549—569.

Sammanfattning

Tillväxten hos *Fomes annosus* odlad på värdväxtmaterial av gran och silvergran.

Inverkan av rot- och stammaterial från gran och silvergran på utvecklingen av *Fomes annosus* undersöktes i laboratorium på tre olika sätt.

1. Odling av rotrötesvampen på sågspånsmedium i Petriskål gav mycket olika resultat. På spån av gran utvecklades ett ytligt mycel vars radietillväxt mättes med dygnsintervall. Tillväxten skedde snabbare i 25°C än i 15°C samt snabbare i spån från rötter än från stamdelar. Hos silvergranspån utvecklades över huvud taget ingen mätbar myceltillväxt i någotdera försöksledet.

2. Finmalet material från 4-åriga plantrötter av gran och silvergran extraherades med destillerat vatten och i det steriliserade extraktet odlades mycel av *F. annosus*. I en koncentrationsserie uppnåddes högsta mycelproduktion i högsta koncentrationen extrakt från gran. I serien med silvergran var förhållandet det omvända, vilket kan tolkas så att hos silvergran en inhibitor för *F. annosus* föreligger, som i låg koncentration tillåter obetydlig myceltillväxt.

3. Utsöndringar — exudat — från rötter av 4-åriga plantor av gran och silvergran sterilfiltrerades och användes som gronings- och tillväxtsubstrat för konidier resp. mycel av rotrötesvampen. Konidietestet utfördes i hängande droppe i fuktig kammare, myceltestet i Petriskål med cellofan-diffusionmetoden. Även i dessa försök blev skillnaden mellan de båda arterna entydig. Ingen groning och ingen myceltillväxt skedde i exudat från *Abies pectinata* medan groning och myceltillväxt i exudat från *Picea abies* närmade sig kontrollernas värden. Den biologiska aktiviteten kunde hänföras till vissa definierade fläckar i en papperskromatografisk analys.